

# Revealing the Secrets of a Deadly Disease

**P**LAGUE is a notorious infectious disease, unrivaled throughout history in its scale of death and devastation. Caused by the enterobacterium *Yersinia pestis*, the disease is carried primarily by rodents (most notably rats) and spreads to humans via fleas. Plague is well known for wreaking havoc on society, such as in the Middle Ages when it was called the Black Death. Yet, even today, plague is endemic in some parts of the world. For example, in 1994, massive flooding from monsoon rains and clogged sewers led to an epidemic in Surat, India, which resulted in 52 deaths.

Not only does *Y. pestis* exist in nature, but it also might be dispersed as a biological weapon. Diagnosing an outbreak of plague could be delayed because the initial symptoms of the disease—headache, weakness, and coughing with hemoptysis (vomiting blood)—are similar to those of other respiratory illnesses. Antibiotics are effective against plague, but without diagnosis and treatment, the infection can be fatal in one to six days.

Researchers do not fully understand how *Y. pestis* infects healthy host cells. To better study its pathogenesis, a team of Livermore scientists in the Physics and Life Sciences Directorate has developed a device that examines these pathogen–host interactions one cell at a time. The system uses nanometer-scale optoelectronic tweezers (OET) to place *Y. pestis* bacteria in contact with susceptible host cells. A miniature camera mounted to a microscope then records the interactions between the pathogen and host as they occur.

Developed with funding from Livermore’s Laboratory Directed Research and Development Program, the new OET system is designed to overcome some limitations of what biomedical scientist Brett Chromy refers to as the “mix-and-hope” method of studying pathogen–host interactions. With that method, researchers grow significant quantities of pathogen cells, mix them with host cells, and look at the combination under a microscope, hoping to observe an interaction. However, because both types of cells are so complex and the specific mechanisms of a pathogen invading a host have yet to be defined, researchers often spend a substantial amount of time scanning for pathogen–host interactions with little success.

According to Chromy, the odds of witnessing one interaction are extremely low, and thousands of bulk measurements are required for a statistically relevant study. The *Y. pestis* cells



Livermore scientist Peter Pauzaskie (above) holds a set of optoelectronic tweezers (OET), a device he helped develop while at the University of California at Berkeley. Below him are the plague bacteria, *Yersinia pestis*, and a common rat—the most notorious carrier for this disease.

measure 1 micrometer in diameter, and the host cells are 20 micrometers in diameter. “At such a small scale, it’s difficult to perform single-cell studies with the mix-and-hope method,” says Chromy, who leads the research team. “So far, no one has been able to zoom in and find a pathogen–host interaction occurring from start to finish. By the time we locate an interaction, we’ve likely missed the first 5 to 10 seconds, which is a critical time for elucidating pathogenesis and the host’s response. With the new OET system, we can observe in real time how the *Y. pestis* cell invades the host cell from the beginning.”

### A Terrestrial Tractor Beam

The nanoscale OET is a low-powered, noninvasive device developed in 2005 by Ming Wu’s electrical engineering group at the University of California (UC) at Berkeley. With this tool, researchers can trap, transport, and sort multiple cells, microparticles, and nanoparticles by projecting optical images onto a glass slide coated with photoconductive materials. OET’s single light-emitting diode can confine more than 10,000 microparticles at one time.

Team member and biophysicist Ted Laurence compares OET to the tractor-beam device in the television show *Star Trek*. “Just as the tractor beam on the Starship Enterprise could trap smaller ships and move them to a different location without direct physical contact,” says Laurence, “OET allows us to transport a *Y. pestis* cell to the location of a host cell.”

One advantage of UC Berkeley’s OET device is its low power requirements. Other cell-manipulation methods, such as optical tweezers, use high-powered lasers. However, the tight focusing requirements of laser beams limit the number of cells that can be moved at one time. OET has an optical power density 100,000 times less than that of optical tweezers, so it can create optical manipulation patterns over large areas in real time.

Another approach for cell manipulation is to create electric fields that either repel or attract particles. Dielectrophoresis, for instance, can move a greater number of particles, but it lacks the resolution and flexibility of optical tweezers. The UC Berkeley team adapted the OET device so that it can also use these dielectrophoretic forces and manipulate numerous cells simultaneously.

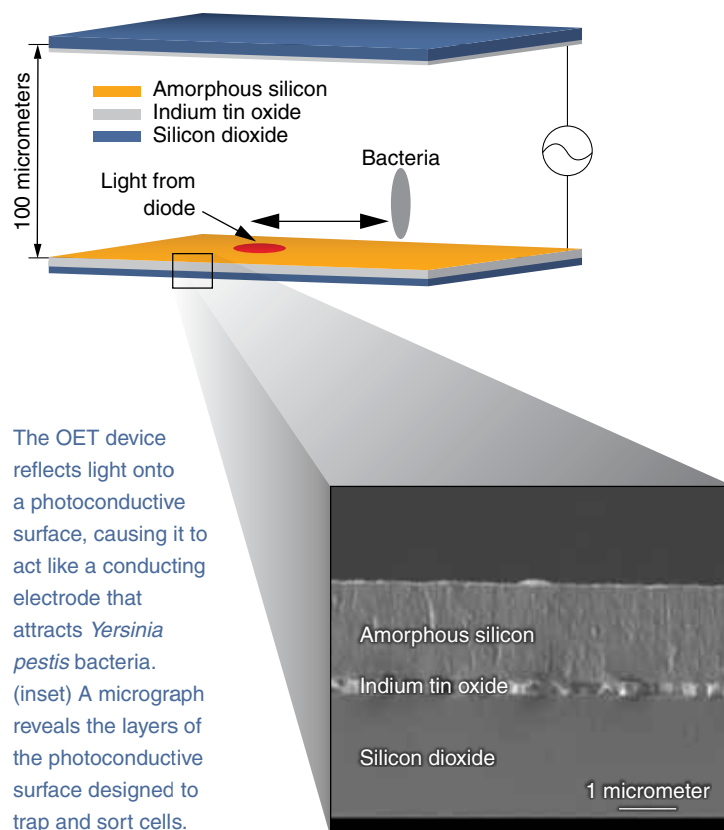
A photoconductive surface made with layers of amorphous or crystalline silicon transforms optical energy into electrical forces. Wherever light hits the photosensitive surface, the material behaves like a conducting electrode, while areas not exposed to light behave like a nonconducting insulator. Once a light source is removed, the material returns to its normal (nonconducting) state.

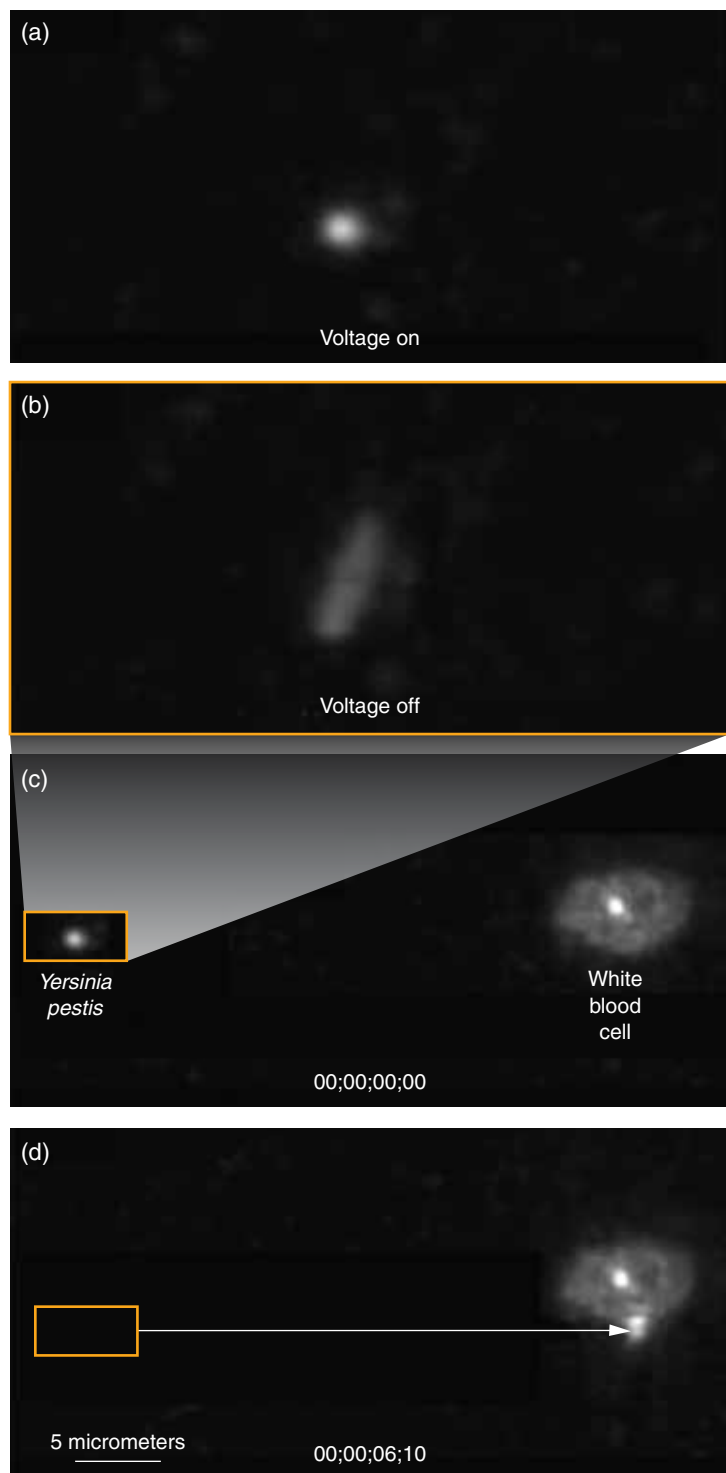
The electric field generated by OET attracts the *Y. pestis* pathogen cells. By adjusting the light pattern, researchers can guide particles to the desired location.

Peter Pauzauskie, a Lawrence fellow who received his doctorate from UC Berkeley, is modifying the OET design for Livermore’s pathogen trapping system. When combined with optical microscopy, the OET device can sort particles or cells based on their luminescence, size, texture, or other visual attribute. For example, a standard technique called dark-field microscopy excludes unscattered light from the image of a *Y. pestis* cell. As a result, the field around the *Y. pestis* cell, which has no specimen to scatter the beam, is generally dark. This technique gives the researchers a clear image of the small *Y. pestis* cells relative to the large host cells.

### Unfolding the Mystery

So far, the OET research team has used the new Livermore system to trap and manipulate fluorescently labeled *Y. pestis* cells





Micrographs of a *Yersinia pestis* cell with voltage (a) on and (b) off show how the bacterium aligns with the electric field created by the two photoconductive surfaces of an OET device. The device can also (c) trap a *Y. pestis* cell and (d) transport it to form a junction with a human white blood cell.

and bring these trapped cells in contact with monocytes—white blood cells in the human immune system. In particular, says Chromy, the team wants to study the interaction between mutant strains of *Y. pestis* and healthy host cells to determine whether the mutant strains are deficient in a protein system called Type III secretion—the main mechanism by which the bacteria infect a host. The team also hopes this research will reveal if certain mutations reduce the ability of *Y. pestis* cells to adhere to a host. Examining interactions between wild-type plague bacteria and monocyte cells engineered to exclude host membrane proteins will also reveal how a host responds to plague.

“The OET trapping system could be used to examine other types of cell interactions,” says Chromy, “for example, to isolate fetal cells in an expectant mother’s blood sample or to sort out abnormally shaped cells from healthy ones. In the future, we want to use the system to study individual plague proteins as they interact with a host cell.” Through this effort, the Livermore researchers will help answer some of the remaining questions about the plague disease.

—Kristen Light

**Key Words:** optoelectronic tweezers (OET), pathogen cells, plague disease, trapping system, *Yersinia pestis*.

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